Appl. No. 10/037,243, Filed January 4, 2002 Amendment Dated April 25, 2005 Reply to Notice of Non-Compliant Amendment of April 19, 2005

Claim Listing This listing of claims will replace all prior versions and listings of claims in the application:

- 1 52: (cancelled)
- (currently amended) An expression vector for enhancing 53. the solubility and proper folding of an expressed protein or polypeptide of interest, said protein or polypeptide having an amino-terminus and a carboxyl-terminus, comprising a first nucleic acid sequence encoding a peptide extension of 61 or fewer amino acid residues, the encoded peptide extension having a net negative charge ranging from -2 to -20 under physiological conditions; the expression vector further comprising a multiple cloning site for inserting, in-frame with said first nucleic acid sequence 10 peptide extension, a second nucleic acid sequence encoding the protein or polypeptide of interest, wherein the expression of the nucleic acid sequences yields a fusion protein consisting essentially of in which the encoded peptide extension—is fused to the carboxyl-terminus of the protein or polypeptide of interest.
  - 54. (original) The vector of Claim 53 which is optimized for use with a prokaryotic cell.

- 55. (original) The vector of Claim 53 which is optimized for use with a eukaryotic cell.
- 56. (currently amended) The expression vector of Claim 53, wherein the net charge of the encoded peptide extension is from -15 to -20.
- 57. (currently amended) The expression vector of Claim 53, wherein the net charge of the <a href="encoded">encoded</a> peptide extension is from -10 to -14.
- 58. (currently amended) The expression vector of Claim 53, wherein the net charge of the <a href="encoded peptide">encoded peptide</a> extension is from -5 to -9.
- 59. (currently amended) The expression vector of Claim 53, wherein the net charge of the encoded peptide extension is from -2 to -4.
- 60. (currently amended) The expression vector of Claim 53, wherein the <u>encoded</u> peptide extension adopts a non-ordered conformation following expression.
- 61. (cancelled)
- 62. (currently amended) The expression vector of Claim 53 wherein the encoded peptide extension comprises the 57 residue—carboxyl-terminal portion—57 amino acid residues of

the a T7 gene 10B protein, or solubility or activity promoting portions thereof.

- 63. (currently amended) The expression vector of Claim 62 wherein one or more of the peptide extension further comprises amino acid substitution variants of the amino acid residues carboxyl terminal portion of the T7 gene 10B protein are substituted, or active portions thereof, which modifications substitutions result in the maintenance of a net negative charge of between -2 and -20 for the encoded peptide extension.
- 64. (currently amended) The expression vector of Claim 62, wherein the encoded peptide extension is selected from the group consisting of: Peptide T7C (SEQ ID NO: 5), Peptide T7B (SEQ ID NO: 6), Peptide T7B1 (SEQ ID NO: 7), Peptide T7B2 (SEQ ID NO: 8), Peptide T7B3 (SEQ ID NO: 9), Peptide T7B5 (SEQ ID NO: 11), Peptide T7B6 (SEQ ID NO: 12), Peptide T7B7 (SEQ ID NO: 13), Peptide T7B8 (SEQ ID NO: 14), Peptide T7B9 (SEQ ID NO: 15), Peptide T7B10 (SEQ ID NO: 16), Peptide T7B11 (SEQ ID NO: 17), Peptide T7B12 (SEQ ID NO: 18), Peptide T7B13 (SEQ ID NO: 19), Peptide T7A1 (SEQ ID NO: 21), Peptide T7A2 (SEQ ID NO: 22), Peptide T7A3 (SEQ ID NO: 21), Peptide T7A3 (SEQ ID NO: 22), Peptide T7A3 (SEQ ID NO: 21), Peptide T7A3 (SEQ ID NO: 22), Peptide T7A3 (SEQ ID NO: 21), Peptide T7A3 (SEQ ID NO: 22), Peptide T7A3 (SEQ ID NO: 21), Peptide T7A3 (SEQ ID NO: 22), Peptide T7A3 (SEQ ID NO: 21), Peptide T7A3 (SEQ ID NO: 22), Peptide T7A3 (SEQ ID NO: 21), Peptide T7A3 (SEQ ID NO: 22), Peptide T7A3 (SEQ ID NO: 21), Peptide T7A3 (SEQ ID NO: 22), Peptide T7A3 (SEQ ID NO: 21), Peptide T7A3 (SEQ ID NO: 22), Peptide T7A3 (SEQ ID NO: 21), Peptide T7A3 (SEQ ID NO: 22), Peptide T7A3 (SEQ ID

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NO: 23), Peptide T7A4 (SEQ ID NO: 24) and Peptide T7A5 (SEQ ID NO: 25).

65 - 86 (cancelled)

- 87. (new) The expression vector of Claim 62 wherein the encoded peptide extension comprises the carboxylterminal 40 amino acid residues of the T7 gene 10B protein.

  88. (new) The expression vector of Claim 87 wherein one or more of the amino acid residues are substituted, which substitutions result in the maintenance of a net negative charge between -2 and -20 for the encoded peptide extension.
- 89. (new) The expression vector or Claim 87 wherein the encoded peptide extension comprises the carboxylterminal 18 amino acid residues of the T7 gene 10B protein and wherein one or more of the amino acids are substituted, which substitutions result in the maintenance of a net negative charge between -4 and -6 for the encoded peptide extension.
- 90. (new) An expression vector for enhancing the solubility and proper folding of an expressed protein or

polypeptide of interest, said protein or polypeptide having an amino-terminus and a carboxyl-terminus, comprising a first nucleic acid sequence encoding a 61 or fewer amino acid peptide extension comprising the carboxyl-terminal 57 amino acid residues of a bacteriophage T7 gene 10B protein, and further comprising a multiple cloning site for inserting, in-frame with said first nucleic acid sequence, a second nucleic acid sequence encoding the protein or polypeptide of interest, wherein expression of the nucleic acid sequences yields a fusion protein consisting essentially of the encoded peptide extension fused to the carboxyl-terminus of the protein or polypeptide of interest.

- 91. (new) The expression vector of Claim 90 wherein one or more of the amino acid residues are substituted, which substitutions result in a net negative charge between -2 and -20 under physiological conditions for the encoded peptide.
- 92. (new) The expression vector of Claim 90 wherein the encoded peptide is selected from the group consisting of: Peptide T7C (SEQ ID NO: 5), Peptide T7B (SEQ ID NO:
- 6), Peptide T7B1 (SEQ ID NO: 7), Peptide T7B2 (SEQ ID NO:

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- 8), Peptide T7B3 (SEQ ID NO: 9), Peptide T7B5 (SEQ ID NO: 11), Peptide T7B6 (SEQ ID NO: 12), Peptide T7B7 (SEQ ID NO: 13), Peptide T7B8 (SEQ ID NO: 14), Peptide T7B9 (SEQ ID NO: 15), Peptide T7B10 (SEQ ID NO: 16), Peptide T7B11 (SEQ ID NO: 17), Peptide T7B12 (SEQ ID NO: 18), Peptide T7B13 (SEQ ID NO: 17), Peptide T7B12 (SEQ ID NO: 18), Peptide T7B13 (SEQ ID NO: 19), Peptide T7A1 (SEQ ID NO: 21), Peptide T7A2 (SEQ ID NO: 22), Peptide T7A3 (SEQ ID NO: 23), Peptide T7A4 (SEQ ID NO: 24) and Peptide T7A5 (SEQ ID NO: 25).
- solubility and proper folding of an expressed protein or polypeptide of interest, said protein or polypeptide having an amino-terminus and a carboxyl-terminus, comprising a first nucleic acid sequence encoding a peptide extension, which peptide extension is selected from the group consisting of: Peptide T7C (SEQ ID NO: 5), Peptide T7B (SEQ ID NO: 6), Peptide T7B1 (SEQ ID NO: 7), Peptide T7B2 (SEQ ID NO: 8), Peptide T7B3 (SEQ ID NO: 9), Peptide T7B5 (SEQ ID NO: 11), Peptide T7B6 (SEQ ID NO: 12), Peptide T7B7 (SEQ ID NO: 13), Peptide T7B8 (SEQ ID NO: 14), Peptide T7B9 (SEQ ID NO: 15), Peptide T7B10 (SEQ ID NO: 16), Peptide T7B11 (SEQ ID NO: 17), Peptide T7B12 (SEQ ID NO: 18), Peptide T7B13 (SEQ ID NO: 19), Peptide T7A1 (SEQ ID NO: 21),

Peptide T7A2 (SEQ ID NO: 22), Peptide T7A3 (SEQ ID NO: 23),

Peptide T7A4 (SEQ ID NO: 24) and Peptide T7A5 (SEQ ID NO:

25), and further comprising a multiple cloning site for

inserting, in-frame with said first nucleic acid sequence,

a second nucleic acid sequence encoding the protein or

20 polypeptide of interest, wherein expression of the nucleic

acid sequences yields a fusion protein consisting

essentially of the encoded peptide extension fused to the

carboxyl-terminus of the protein or polypeptide of

interest.